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EXAMINER	
FORD, ALLISON M	
ART UNIT	**PAPER NUMBER**
1651	

DATE MAILED: 09/27/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/815,388	CAVIEDES ET AL.	
	Examiner	Art Unit	
	Allison M. Ford	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 July 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-3,8,15 and 18-32* is/are pending in the application.

4a) Of the above claim(s) *1-3,8,15 and 32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *18-31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *31 March 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election of Group III, claims 18-20, in the reply filed on 22 July 2005 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)). Applicants have submitted new claims 21-32. Claims 21-31 are directed to the cell culture of original group III; however, newly submitted claim 32 is directed to an invention that is independent or distinct from the invention originally claimed for the following reasons: new claim 32 is directed to a method of making the cell culture of claim 18; thus the claims are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make other and materially different product or (2) that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the instant case culturing the one or more process forming cells in the presence of agents that would inhibit adhesion, such as polyvinyl alcohol, can alternatively make the cell culture of claim 18. Thus one could produce the culture of claim 18 by alternative methods than that of claim 32.

Since applicant has received an action on the merits for the originally presented invention, this invention has been constructively elected by original presentation for prosecution on the merits. Accordingly, claim 32 is withdrawn from consideration as being directed to a non-elected invention. See 37 CFR 1.142(b) and MPEP § 821.03. This restriction requirement is made FINAL.

Status of the Application

Claims 1-3, 8, 15, and 18-32 are pending in the current application, with claims 1-3, 8, 15 and 32 being withdrawn from consideration. Claims 18-31 have been examined on the merits.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 18-31 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicant's claims are directed to a cell culture comprising one or more process-forming cells in the absence of cell attachment treatments or cell attachment factors. Applicant's specification defines "cell attachment treatments" as any physical and/or chemical treatment or conditioning of a substrate that promotes passive or active adhesion of a cell to a substrate surface (See Spec Pg. 4); specific examples include microfluidic networks that deliver adhesive proteins or live cells directly to the substrate, or application of plasma deposited polymer, derived from acrylic acid (See Spec. Pg. 5). Applicant's specification defines "cell attachment factors" as any molecule or chemical moiety that promotes passive or active adhesion of a cell to a substrate surface. Substrates that lack cell attachment factors (not formulated, coated or associated with cell attachment factors (See Spec, Pg. 4); specific examples include RGD cell attachment sequence (human fibronectin).

Applicant fails to provide sufficient written description of cell attachment factors or cell attachment treatments, much less sufficient description of a representative number of species which is required to claim the entire genuses of cell attachment factors and cell attachment treatments. The definitions provided by applicant only disclose the functional property of the cell attachment factors and treatments (factors, or application of factors, that promote passive or active adhesion of a cell to a substrate surface); the mere disclosure of the action is not sufficient to show the applicant was in

possession of the claimed genus. The only specific example of a cell attachment factor that applicant has provided is the RGD cell attachment sequence (human fibronectin) (Spec, Pg. 5-6); the other examples, which include adhesive proteins, live cells, or plasma deposited polymers, are so broad and non-specific that one cannot determine any shared, relevant, identifying characteristics, such as structure or other physical or chemical properties, or functional characteristics, beyond disclosure of the generic action (promoting adhesion), within the group. Because applicant's invention relies on the exclusion of such factors or treatments, one must be able to specifically define and determine what factors/treatments fall into the genus. *See Eli Lilly,* 119F. 3d. at 1568, 43 USPQ2d at 1406. See MPEP § 2163.

Claims 20, 25 and 29 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a cell culture comprising one or more process-forming neuronal cells, wherein there is substantially no attachment of the neuronal cells to the substrate, does not reasonably provide enablement for a cell culture comprising any type of process-forming cells, wherein there is substantially no attachment of the neuronal cells to the substrate. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

Applicant's claims are directed to a cell culture comprising one or more process-forming cells in the absence of cell attachment treatments or cell attachment factors, wherein there is substantially no attachment of the process-forming cells to the substrate.

Applicants' provide a single working example in the specification wherein RCSN-3 cells (rat neuronal cells from the substantial nigra) were seeded on polystyrene microbiological plates that were not tissue culture treated; the authors stated spheroid-like cell aggregates that lacked processes were obtained (See Spec. Pg, 19). According the disclosure, it is the absence of cell attachment treatments or cell attachment factors on the polystyrene microbiological plates that prevented attachment to produce

spheroid-like cell aggregates that lacked processes. Applicant's claims are directed to cultures of all process-forming cells, claim 27 specifically requires the process forming cells can be glial cells, muscle cells, connective tissue cells, and endothelial cells; however applicant has provided no working examples using cell types other than neurons (RCSN-3 cells).

Enablement is lacking for *all* cultures of *all* process-forming cells, wherein there is substantially no attachment of the process-forming cells to the substrate, because the prior art provides teachings wherein process-forming cells cultured on untreated polystyrene do adhere and attach, which specifically teaches away from the claimed invention. Specifically Grinstaff et al (US 2003/0185870) cultures human fibroblasts and umbilical vein endothelial cells on untreated polystyrene; Grinstaff et al report that the cells lose their rounded morphology and adhere to the untreated control plates (See Pg. 25-26, paragraphs 0318-0319). Therefore applicant is not enabled for cell cultures comprising any type of process-forming cells in the absence of cell attachment treatments or cell attachment factors, wherein there is substantially no attachment of the process-forming cells to the substrate.

While a singular, narrow working embodiment cannot be a sole factor in determining enablement, its limited showing, in light of the negative teachings in the art and the lack of description and guidance present in the application, provides additional weight to the lack of enablement in consideration of the *Wands* factors as a whole. Thus, one of ordinary skill in the art would not have a reasonable expectation of success of creating a cell culture comprising any type of process-forming cells, wherein there is substantially no attachment of the neuronal cells to the substrate, as in the claimed invention.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 18-31 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicant's claim 18 is directed to a cell culture comprising one or more process-forming cells in the absence of cell attachment treatments or cell attachment factors. It is unclear, however, what applicant is defining as "cell attachment treatments" or "cell attachment factors." Even in light of the specification one cannot determine what molecules or factors are to be considered "cell attachment factors" applied during a "cell attachment treatment;" therefore one of ordinary skill in the art cannot determine the metes and bounds of the claimed subject matter as they cannot determine what, specifically, is being claimed.

The term "substantially" in claim 20 is a relative term which renders the claim indefinite. The term "substantially" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Therefore the claim is rendered indefinite because one skilled in the art cannot determine the metes and bounds of the claimed subject matter. Additionally, it is not clear how the solid substrate supports the process-forming cells if there is no attachment of the process-forming cells to the substrate. Rather it would appear that the process-forming cells are not supported by any structure, but are maintained in suspension.

Again, the term "substantially" in claim 25 is a relative term which renders the claim indefinite. The term "substantially" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Therefore the claim is rendered indefinite because one skilled in the art cannot determine the metes and bounds of the claimed subject matter. Additionally, there is insufficient antecedent basis for the limitation "said solid substrate" in parent claim 18; it is not clear if claim 25 is to depend from 18 or from 21.

Applicant's claim 26 requires the cell culture of claim 18 to have a calcium concentration of 50 um or less. Micrometers (um) is not a unit of concentration, it appears applicant intended for the claim to require the calcium concentration to be 50 uM or less. Examination has been conducted as such.

Applicant's claim 29 is directed to the cell culture of claim 18, wherein said process-forming cells are clustered, three-dimensional aggregates. It is not clear from the claim if the process-forming cell aggregates can be attached to the substrate or not. It is noted that it is not required in claim 18 that the cells not be attached to any substrate; therefore it is not clear if the aggregates must be in suspension, or if they can be attached.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 18, 21-22, 24, and 29-31 are rejected under 35 U.S.C. 102(b) as being anticipated by Steinman et al (US Patent 5,994,126).

Applicant's claim 18 is directed to a cell culture comprising one or more process-forming cells in the absence of cell attachment treatments or cell attachment factors. Claim 21 requires the cell culture to further comprise a solid substrate supporting said process-forming cells, wherein the substrate has not been treated to promote cell attachment and lacks cell attachment factors that would promote adhesion of

the process-forming cells thereto. Claim 22 requires the solid substrate to be a culture vessel selected from a Petri dish, a flask, a bottle, a plate, a tube, and a vial. Claim 24 requires the solid substrate to be a microbiological plate. Claim 29 requires the process-forming cells to be clustered, forming three-dimensional aggregates. Claim 30 requires the cell culture to comprise two or more types of process-forming cells. Claim 31 requires the cell culture to further comprise non-process forming cells.

Steinman et al teach cultures of dendritic cells (which applicant calls process-forming cells) derived from human blood. Steinman et al produce the cultures by co-culturing non-adherent aggregates of dendritic precursor cells with adherent, non-process-forming fibroblasts and macrophages. Steinman et al utilize culture flasks (a microbiological plate) as the solid surface on which the monolayer of fibroblasts and macrophages forms. The non-adherent cell aggregates loosely adhere to the fibroblast and macrophage monolayer; but remain substantially unadhered to the culture flask surface (See col. 17, ln 59-col. 18, ln 31). Thus Steinman et al produce a cell culture comprising dendritic precursor cells (which will become mature dendritic cells, and thus are process-forming cells) and non-process-forming fibroblasts and macrophages in a culture flask (which applicant calls a solid substrate), wherein the dendritic precursor cells cluster to form three-dimensional aggregates that loosely adhere to the fibroblast and macrophage monolayer, but are substantially unattached to the solid substrate (Claims 18, 21, 22, 24, 25, 29, and 31).

Steinman et al further teach dislodging the aggregates and subculturing (inherently in a culture flask) the aggregates on a separate substrate (subculturing implies the cell aggregates are cultured in a culture flask) to which the aggregates do not substantially adhere. Steinman et al teach that after 10-17 days in this subculture the aggregate will contain both mature dendritic cells and dendritic precursor cells (wherein both mature dendritic cells and precursor dendritic cells are process-forming cells) (See col. 17, ln 59-col. 18, ln 31). Thus Steinman et al teach a cell culture of comprising two different types of process-forming cells in clustered, three-dimensional aggregates (mature dendritic cells and precursor

dendritic cells), in the absence of cell attachment treatment or cell attachment factors (Claims 18, 21, 22, 24, 25, 29 and 30).

Therefore the reference anticipates the claimed subject matter.

Claims 18, 21-25 and 30 are rejected under 35 U.S.C. 102(b) as being anticipated by Weiss et al (US Patent 5,981,165).

Applicant's claim 18 is directed to a cell culture comprising one or more process-forming cells in the absence of cell attachment treatments or cell attachment factors. Claim 21 requires the cell culture to further comprise a solid substrate supporting said process-forming cells, wherein the substrate has not been treated to promote cell attachment and lacks cell attachment factors that would promote adhesion of the process-forming cells thereto. Claim 22 requires the solid substrate to be a culture vessel selected from a Petri dish, a flask, a bottle, a plate, a tube, and a vial. Claim 23 requires the solid substrate to comprise untreated plastic. Claim 24 requires the solid substrate to be a microbiological plate. Claim 25 requires there to be substantially no attachment of said process-forming cells to said solid substrate. Claim 30 requires the cell culture to comprise two or more types of process forming cells.

Weiss et al teach a method of forming a cell culture comprising tyrosine-hydroxylase expressing cells (neural process-forming cells), and the cell culture produced (See claims 1 and 21). Weiss et al teach that cell culture can be grown in any suitable container, including a tissue culture flask, well, or Petri dish. Weiss et al teach that when adherence is not desired, glass or untreated plastic tissue culture substrates can be used (which applicant calls untreated microbiological plates) (See col. 7, ln 25-46); thus when glass or untreated plastic tissue culture substrates are used the cell culture comprises tyrosine-hydroxylase expressing cells (process-forming neural cells) and an untreated solid plastic substrate, wherein no cell attachment treatments or cell attachment factors are contained in the culture or on the solid substrate, so that the cells do not adhere (Claims 18, 21-25, 28). Weiss et al also teach that a

monolayer of feeder cells, comprising neurons, astrocytes or oligodendrocytes (all process-forming cells) can be included in a co-culture (See col. 7, ln 41-46) (Claim 30). Therefore the reference anticipates the claimed subject matter.

Claims 18, 19, 21, 25 and 27-29 rejected under 35 U.S.C. 102(b) as being anticipated by Takazawa et al (US Patent 5,219,752).

Applicant's claim 18 is directed to a cell culture comprising one or more process-forming cells in the absence of cell attachment treatments or cell attachment factors. Claim 19 requires the cell culture to be free of calcium or contain a low concentration of calcium. Claim 21 requires the cell culture to further comprise a solid substrate supporting said process-forming cells, wherein the substrate has not been treated to promote cell attachment and lacks cell attachment factors that would promote adhesion of the process-forming cells thereto. Claim 25 requires there to be substantially no attachment of said process-forming cells to said solid substrate. Claim 27 requires the process forming cells to be selected from the group consisting of glial cells, muscle cells, connective tissue cells, and endothelial cells. Claim 28 requires the process-forming cells to be neurons. Claim 29 requires the process-forming cells to be clustered, forming three-dimensional aggregates.

Takazawa et al teach an animal cell culture wherein adherent animal cells are cultured in such conditions so that the cells do not adhere, but remain in suspension as single cells or small cell clumps. Takazawa et al teach a wide variety of normally adherent animal cells can be maintained in suspension culture by the means of their invention, the types of cells include rat glial cells (ATCC No. CCL 107); mouse muscle cells (ATCC Nos. CRL 1443, CCL 198, CRL 1447, CRL 1456, CCL 197); mouse connective tissue cells (ATCC Nos. CCL 1.2, CCL 1.3, CCL 1, CCL 11, CCL 12); bovine endothelial cells (ATCC Nos. CCL 207, CCL 209); and mouse neuroblastomas (neurons) (ATCC No. CCL 131) (See Table spanning col. 5-12). The cell culture of Takazawa et al further includes serum-free media with a

calcium ion (Ca^{2+}) concentration of 0.002 mM to 0.3 mM (2uM to 300uM), preferably 0.02 mM to 0.25mM (20uM to 250uM) (See col. 13, ln 66-col. 14, ln 17). The cell culture of Takazawa et al further comprised a cell culture vessel (which applicant calls a solid substrate) (See Fig. 1). No cell attachment treatments or cell attachment factors were included. Takazawa et al teach the cells remain in suspension as single cells, or in small cell aggregates of 1.1-50 cells on average (which applicant calls not substantially adhering to the substrate) (See col. 14, ln 33-43) (Claims 18, 19, 21, 25 and 27-29). Therefore the reference anticipates the claimed subject matter.

Claims 18, 21-24 and 27 rejected under 35 U.S.C. 102(e) as being anticipated by Grinstaff et al (US 2003/0185870 A1).

Applicant's claim 18 is directed to a cell culture comprising one or more process-forming cells in the absence of cell attachment treatments or cell attachment factors. Claim 21 requires the cell culture to further comprise a solid substrate supporting said process-forming cells, wherein the substrate has not been treated to promote cell attachment and lacks cell attachment factors that would promote adhesion of the process-forming cells thereto. Claim 22 requires the solid substrate to be a culture vessel selected from a Petri dish, a flask, a bottle, a plate, a tube, and a vial. Claim 23 requires the solid substrate to comprise untreated plastic. Claim 24 requires the solid substrate to be a microbiological plate. Claim 27 requires the process-forming cells to be selected from glial cells, muscle cells, connective tissue cells, and endothelial cells.

Grinstaff et al separately culture adult human dermal fibroblasts (NHDFs) (connective tissue cells) and human umbilical vein endothelial cells (HUVECs) (endothelial cells) on untreated polystyrene culture plates (which applicant calls microbiological plates, also solid surfaces). Both cell types adhered to the untreated polystyrene plates after overnight culture (See Pg. 26, paragraph 0318-0319). Grinstaff et al thus produced cultures of fibroblasts and endothelial cells (process-forming cells), wherein the solid,

untreated, plastic substrate supported the cells (Claims 18, 21-24 and 27). Therefore the reference anticipates the claimed subject matter.

Claims 18-26 and 28-29 are rejected under 35 U.S.C. 102(a) as being anticipated by Andrews et al (Poster presentation from Cell Culture and Engineering Conference in Snowmass, CO; 2002).

Applicant's claim 18 is directed to a cell culture comprising one or more process-forming cells in the absence of cell attachment treatments or cell attachment factors. Claim 19 requires the cell culture to be free of calcium or contain a low concentration of calcium. Claim 20 requires the cell culture to further ocmprise a solid substrate supporting the process-forming cells, wherein there is substantially no attachment of said process-forming cells to said substrate, and wherein said cell culture has a calcium concentration of 100 uM or less. Claim 21 requires the cell culture to further comprise a solid substrate supporting said process-forming cells, wherein the substrate has not been treated to promote cell attachment and lacks cell attachment factors that would promote adhesion of the process-forming cells thereto. Claim 22 requires the solid substrate to be a culture vessel selected from a Petri dish, a flask, a bottle, a plate, a tube, and a vial. Claim 23 requires the solid substrate to comprise untreated plastic. Claim 24 requires the solid substrate to be a microbiological plate. Claim 25 requires there to be substantially no attachment of said process-forming cells to said solid substrate. Claim 26 requires the cell culture to have a calcium concentration of 50 uM or less. Claim 28 requires the process-forming cells to be neurons. Claim 29 requires the process-forming cells to be clustered, forming three-dimensional aggregates.

Andrews et al teach culturing RCSN-3 cells (neurons) in microbiological plates (which applicant calls a solid substrate which support the neuronal cell growth) to produce a mass suspension culture wherein the cells do not adhere to the substrate, but form three-dimensional aggregates (Claims 18, 21, 22, 24, 25, 28 and 29). The microbiological plates were plastic, and were untreated by any cell

attachment treatments or cell attachment factors (as evidenced by their comparison to 'standard conditions' comprising glass or plastic treated plates) (Claim 23). Andrews et al teach the neuronal cells were maintained in an optimized media comprising low serum (2%) supplemented with hormones (insulin, progesterone), proteins (transferrin), and trace elements (Sodium selenite, putrescine). Andrews et al is silent on the calcium concentration of this media; however, it appears to be the same media used in the present application (See Spec, Pg. 19), and thus has the same low calcium concentration (less than 50 uM) as in the current application (Claims 19, 20, and 26).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 20, 22-24, 26, and 30-31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Takazawa et al (US Patent 5,219,752).

Applicant's claims are directed to a cell culture comprising one or more process-forming cells in the absence of cell attachment treatments or cell attachment factors. Claim 20 requires the cell culture to further ocmprise a solid substrate supporting the process-forming cells, wherein there is substantially no attachment of said process-forming cells to said substrate, and wherein said cell culture has a calcium concentration of 100 uM or less. Claim 22 requires the solid substrate to be a culture vessel selected from a Petri dish, a flask, a bottle, a plate, a tube, and a vial. Claim 23 requires the solid substrate to comprise untreated plastic. Claim 24 requires the solid substrate to be a microbiological plate. Claim 26 requires the cell culture to have a calcium concentration of 50 uM or less. Claim 30 requires the cell culture to

comprise two or more types of process-forming cells. Claim 31 requires the cell culture to further comprise non-process forming cells.

Takazawa et al teach an animal cell culture wherein adherent animal cells are cultured in such conditions so that the cells do not adhere, but remain in suspension as single cells or small cell clumps. Takazawa et al teach a wide variety of normally adherent animal cells can be maintained in suspension culture by the means of their invention, the types of cells include rat glial cells (ATCC No. CCL 107); mouse muscle cells (ATCC Nos. CRL 1443, CCL 198, CRL 1447, CRL 1456, CCL 197); mouse connective tissue cells (ATCC Nos. CCL 1.2, CCL 1.3, CCL 1, CCL 11, CCL 12); bovine endothelial cells (ATCC Nos. CCL 207, CCL 209); and mouse neuroblastomas (neurons) (ATCC No. CCL 131) (See Table spanning col. 5-12). The cell culture of Takazawa et al further includes serum-free media with a calcium ion (Ca^{2+}) concentration of 0.002 mM to 0.3 mM (2uM to 300uM), preferably 0.02 mM to 0.25mM (20uM to 250uM) (See col. 13, ln 66-col. 14, ln 17). The cell culture of Takazawa et al further comprised a cell culture vessel (which applicant calls a solid substrate) (See Fig. 1). No cell attachment treatments or cell attachment factors are included. Takazawa et al teach the cells remain in suspension as single cells, or in small cell aggregates of 1.1-50 cells on average (which applicant calls not substantially adhering to the substrate) (See col. 14, ln 33-43).

Though the concentration of calcium ions taught by Takazawa et al has a higher upper limit than that which is currently claimed, at the time of the claimed invention, it would have been well within the purview of one of ordinary skill in the art to optimize the amount calcium ion to be included in the cell culture of Takazawa et al as a matter of routine experimentation (Claims 20 and 26). Moreover, at the time of the claimed invention, one of ordinary skill in the art would have been motivated by routine practice to optimize the concentration of calcium ions in the cell culture with a reasonable expectation for successfully obtaining a cell culture that can effectively produce active biological agents secreted from the cultured cells in a higher quantity than possible in adherent cultures within the same culture vessel.

Generally, differences in concentration will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical or produces unexpected results. Where the general conditions of a claim are disclosed by the prior art it is not inventive to discover the optimum or workable ranges by routine experimentation, See *In re Aller,* 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). Specifically note that where the claimed ranges overlap, such as in the instant case, or lie inside ranges disclosed by the prior art a prima facie case of obviousness exists. See *In re Wertheim,* 541 F.2d 257, 191 USPQ 90 (CCPA 1976); *In re Woodruff,* 919 F.2d 1575, 16 USPQ2d 1934 (Fed. Cir. 1990). Therefore, though Takazawa et al claim a calcium ion concentration of 0.002mM to 0.3mM (2uM to 300uM), preferably 0.02mM to 0.25mM (20uM to 250uM) Ca^{2+}; it would have been prima facie obvious to optimize this concentration to below 100uM or 50uM.

Furthermore, though Takazawa et al disclose a culture vessel (which applicant calls a solid substrate) and provide a drawing of the culture device, they are silent on the exact material of the culture vessel. The culture vessel of Takazawa et al was designed to automatically cycle the culture medium to and from the cell culture; however, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use a standard, microbiological grade culture vessel, such as a Petri dish, flask, bottle, plate, tube, or vial (Claims 22 and 24). It would further have been obvious to one of ordinary skill in the art to use a standard, microbiological grade Petri dish, flask, bottle, tube, plate or vial consisting of untreated plastic (Claim 23), in order to prevent adhesion of the cells to the substrate. One of ordinary skill in the art would have been motivated to use any form of a microbiological grade culture dish because these types of culture vessels are the standard used in cell culture and are available from a variety of laboratory product retailers. One would further have been motivated to use such basic culture vessels instead of the automated device of Takazawa et al in order to save money, as an automated machine would be more expensive, and in order to be able to change the culture medium according to the growth rate of the cells, as opposed to a scheduled media change as is done by automated systems. One

of ordinary skill in the art would have been motivated to use untreated microbiological grade (as opposed to tissue culture grade) culture vessels so as to prevent/reduce cell adhesion to the solid substrates; as Takazawa et al desire for the cells to not adhere in a monolayer, but stay in a mass suspension (See col. 1, ln 55-col. 2, ln 16). One would expect success using any suitable, untreated, microbiological grade culture dish to create the cell culture of Takazawa et al because the cells of Takazawa et al are not intended to adhere to the substrate, but rather to be prevented from adhering based on the low concentration of calcium. Additionally, because adhesion is not desired, one would expect successfully decreasing adhesion by using culture dishes not treated to promote adhesion.

Finally, though Takazawa et al do not teach co-culturing two or more types of cells, wherein the second cell types either are also process-forming cells, or are non-process-forming cells, it would have been obvious to one skilled in the art at the time the invention was made to include multiple cell types that are capable of growing in the same general culture conditions in order to obtain a mixture of bioactive products from two or more cell types (Claims 30 and 31). Takazawa et al create the suspension culture in order to produce biologically active substances, secreted by the cells, in higher concentrations than permitted by adherent culture, based on the fact that more cells per volume can be maintained in suspension culture than can be maintained in adherent culture (See col. 4, ln 37-60). Thus by culturing two or more different cell types together, the collected culture medium will comprise a mixture of growth factors and proteins that can subsequently be used for future cell culture. By culturing two or more cell types together one saves the time and energy on culturing two separate cell populations and then combining the biologically active products obtained therefrom. One of ordinary skill in the art would be motivated to use mixtures including two or more types of process-forming cells or one type of process-forming cell and one type of non-process forming cell in order to create different cocktails of growth mixtures and hormones that would be useful for future cell cultures and experimentations. One would expect success because co-culturing cells is well known in the art; one of ordinary skill would be able to

select two or more cell types that could successfully be cultured together with a reasonable expectation of successfully obtaining a biologically active substance containing a combination of growth factors and other secreted proteins and hormones from each cell type. Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Allison M. Ford whose telephone number is 571-272-2936. The examiner can normally be reached on 7:30-5 M-Th, alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Allison M Ford
Examiner
Art Unit 1651

LEON B. LANKFORD, JR.
PRIMARY EXAMINER